Ventilation and Microbial Communities in LEED-Certified Buildings

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Abstract

For over a decade and a half, the U.S. Green Building Council (USGBC) has strived to improve building efficiency and indoor environmental quality around the world through its Leadership in Energy and Environmental Design (LEED) certification program. Attempts to maximize energy efficiency in LEED-certification projects may be in conflict with indoor air quality with regards to microbial communities if building designs employ lower ventilation rates in order to reduce energy demand. However, the influence of LEED certification on indoor microbial communities is largely unknown. The aim of this study is to investigate the effects that LEED design principles have on indoor microbial communities. We collected carpet dust and suspended air dust at six paired LEED and non-LEED buildings, extracted DNA from these samples and then analyzed them by qPCR and high-throughput DNA sequencing. Sequencing results were analyzed using QIIME version 1.9 and compared to air exchange rates. Results indicated that air exchange rates were significantly lower in LEED compared to non-LEED buildings. Consequentially, no significant correlations were seen between air exchange rates and microbial communities. Sequencing results were also used to determine the diversities of the microbial communities, along with the similarity of communities in carpet dust with that of indoor and representative outdoor air. Non-LEED buildings demonstrated bacterial communities more similar to that of outdoor air than LEED for both weighted and unweighted UniFracs. However, results were not consistent for all building pairs in either weighted or unweighted UniFrac distance comparisons. Bacterial communities in non-LEED carpets were consistently more similar to all related air samples than in LEED for the unweighted UniFrac, but this correlation was not statistically significant. Fungal communities in non-LEED entrance and backroom carpet samples were more different than in LEED, demonstrated by the Morisita-Horn distance comparison, but no significant correlation was found. These results demonstrated that LEED certification may influence indoor microbial communities, and mechanisms and effects will need to be determined in future studies.

Introduction

In 2000, the non-profit organization U.S. Green Building Council (USGBC) established the Leadership in Energy and Environmental Design (LEED) building certification program. According to USGBC, LEED is "the most widely recognized and widely used green building program across the globe".[1]

There is a growing number of LEED-certified buildings, making claims of improved energy efficiency and occupant living conditions among other attractive benefits. This raises interest in the relationships between potentially competing LEED standards. Though LEED design principals are holistic in theory, only 16 of the 110 points (14.5%) used to determine a building's LEED rating for building design and construction projects are awarded for indoor environmental quality. Of these, only 5 (4.5%) deal directly with air quality as it relates to human health. On the other hand, 33 points (30%) are designated for energy and atmosphere.[2] LEED ratings strongly prioritize energy use while occupant health risks from indoor air quality are given less consideration.

In order to earn more points in the energy and atmosphere area, LEED projects could reduce energy demand from heating, ventilation and air conditioning (HVAC) infrastructure by lowering ventilation rates, providing they meet the current minimum ventilation standards set by the American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE).[2] This reduction in ventilation could cause both chemical and biological air pollutants to be trapped in the indoor environment, impacting human health and productivity alike.[3] Indoor ventilation rates can have a significant influence both on the extent to which indoor are similar to outdoor bioaerosols,[4] and on the prevalence of asthma and allergy symptoms, and sick building syndrome (SBS) among occupants. Higher than average ventilation rates are desirable for improving the overall air quality of the indoor environment.[5] In fact, the most significant factors that influence indoor microbial communities are ventilation and occupancy.[6] By quantifying ventilation rates and comparing

microbial communities in LEED-certified buildings, the goal of this research was to determine how LEED certification may influence the indoor microbiome and human exposure.

Methodology

Building Selection

Air and dust samples from 3 LEED certified and 3 non-LEED buildings on the campus of The Ohio State University were collected during the summer and fall of 2016. Only buildings without evidence of mold or water leaks and chemical or biological laboratories were selected. Other contributing factors such as occupancy, air conditioning use, and location on campus were also considered in the building selection process. Samples were collected during hours of building occupancy. Buildings were paired together by like floor area, use and occupancy. Pair 1 consisted of two large classroom buildings with small theaters in each used for the backroom carpet sampling. Pairs 2 and 3 were residential buildings. LEED-certification levels were provided for each LEED-certified building.

Air Sampling

Air sampling was completed by use of SKC Button Samplers (SKC, Eighty Four, PA, USA) fitted with 25mm diameter, 0.8μm pore-size mixed cellulose ester (MCE) filters (Advantac MFS, Inc., Dublin, CA, USA) through which air was passed at an average of 4 L/min via a SKC 224 (SKC, Eighty Four, PA, USA) or SKC QuickTake30 (SKC, Eighty Four, PA, USA) pump. Two samples were taken in each building: 1) located near the building's entrance; 2) located either immediately outside or immediately inside of the building's air-intake corresponding to the entrance area. Paired buildings were sampled simultaneously for a continuous 24 hours. Ambient temperature and relative humidity (RH) were recorded at each sampling site using a HOBO UX100-003 temperature and relative humidity logger (Onset Computer Corp., Cape Cod, MA, USA). Following sample collection, filters were stored at -20°C until DNA isolation.

Dust Sampling

Dust samples were collected immediately before and immediately after air sampling from high-traffic areas within each building using a Eureka Mighty Mite vacuum (Eureka Co., Bloomington, IL,

USA) fitted with a 19 × 90mm Whatman cellulose extraction thimble (Whatman, Inc., Tewkesbury, MA, USA). A standardized protocol was followed sampling a 1m^2 floor section for a duration of 2 minutes.[7,8]

Ventilation

Air exchange rates (AERs) were calculated by emitting non-toxic concentrations of carbon dioxide (CO_2) as a tracer gas in the entrance area of each building and measuring CO_2 decay with a HOBO MX1102 CO_2 logger (Onset Computer Corp., Cape Cod, MA, USA). CO_2 concentrations measured at 1 second intervals were plotted over time. **Equation 1** was used to determine the average AER during the decay portion of the experiment.[9] CO_2 plots with trendlines made using the calculated AERs are shown in **Table 10** of the Appendix.

Equation 1: $C(t) - C_{ext} = (C_0 - C_{ext}) \times e^{[-AER(t-t_0)]}$

For one experiment, two CO_2 loggers were used and positioned on opposite sides of the room to verify complete mixing of carbon dioxide in the room. Air exchange rates derived from this test deviated by less than 4% from their average (**Table 1**).

DNA Extraction and Sequencing

A one-half section of the sampled MCE filter or a 250 mg aliquot of unsieved dust samples were used for the DNA extraction. In cases where 250 mg of dust was not available, one-half of the available dust was used during DNA extraction and the quantity used recorded. The PowerLyzer® PowerSoil® DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA, USA) was employed to complete the extraction. Nucleic acids from extracted air samples were purified according to the kit protocol following bead beating, then eluted using a 50mL TE buffer.

Taxonomic libraries of fungi were made with ITS1F and ITS4 primers thus amplifying the internal transcribed spacer (ITS) region.[10] For bacteria, 515F/ 806R primers were used to sequence the V4

region of 16S rRNA. Samples were sequenced using the Illumina® MiSeq with 2x300 bp chemistry (Illumina® Inc., San Diego, CA, USA).

DNA Sequence Analysis

QIIME version 1.9 was used to analyze diversity of microbial data for quality trimming, denoising, and clustering at 97% similarity. Paired-end reads were joined and samples were filtered to a Phred score of 20. Distance matrix comparisons and principal coordinate analyses were used to determine associations between building characteristics and similarities in microbial communities using the Morisita Horn distance for fungi and both the weighted and unweighted UniFrac distance for bacteria. Specifically, indoor air was compared to floor dust and outdoor air, and entrance carpet was compared to backroom carpet in both LEED-certified and non-LEED buildings.

Distance comparisons relate phylogenetic distances between organisms observed within the communities. A smaller distance indicates more genetic similarity between communities, whereas a larger distance is indicative of more genetic diversity between communities. Principle coordinate analyses (PCoAs) are provided to visualize distance matrices where multiple communities are considered. Units on both axes of PCoAs are arbitrary. Communities are more similar if they are closer together on PCoA plots, and more different if they are farther apart.

Results

Ventilation

Ventilation rates for non-LEED buildings were not significantly different from that of corresponding LEED buildings ($R = 0.09$, $P = 0.87$, $P > 0.05$). Pair 3 demonstrated a substantially lower air-exchange rate in the non-LEED building compared to LEED (**Table 1**).

Table 1: AERs for each building calculated using tracer CO_2 concentrations and Equation 1.

	Building Type	AER (hr^{-1})	STD
Pair 1	LEED	7.74	-
	non-LEED	9.38	-
Pair 2	LEED	8.46	-
	non-LEED	10.38	-
Pair 3	LEED	9.88	-
	non-LEED	5.38	0.05

Bacterial Sequencing

We included a total of 550958 bacterial reads in this analysis. We used weighted and unweighted UniFrac distances to compare community similarities. The weighted UniFrac distance accounts for operational taxonomic unit (OTU) abundance in determining the distance between bacterial communities, meaning total sample quantity is important for determining the weighted UniFrac distance between samples. Only samples of similar type and extraction quantity should be considered in weighted UniFrac comparisons. The unweighted UniFrac distance does not depend upon OTU abundance. Neither the weighted nor unweighted UniFrac distance for bacteria from indoor to outdoor air samples correlate to the building's AER ($P > 0.05$, **Figure 1**).

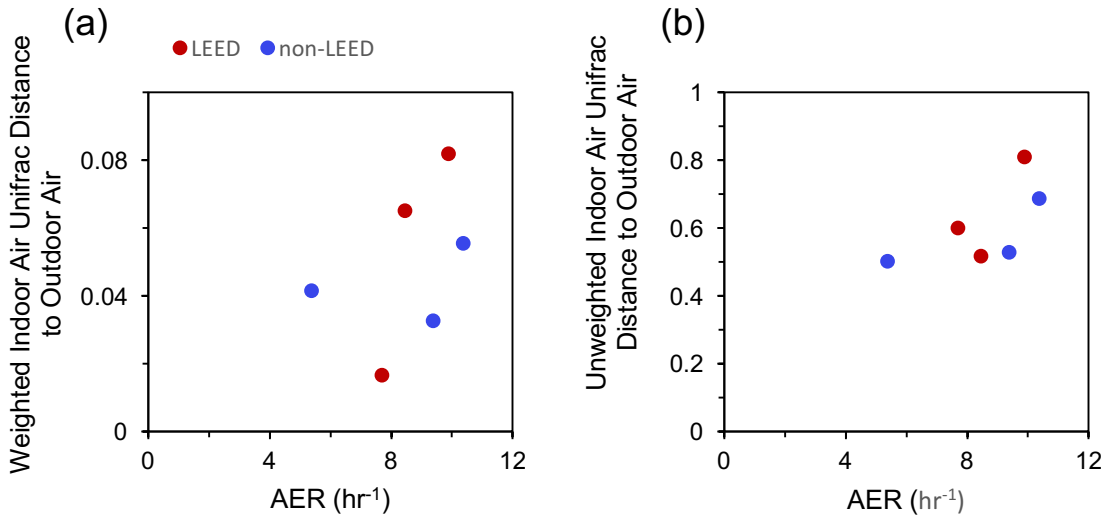

Figure 1: (a) Weighted and (b) unweighted UniFrac distance of indoor to outdoor air compared to AER. (a) R = 0.43, P = 0.39. (b) R = 0.32, P = 0.54.

Relative humidity varied between LEED and non-LEED buildings. No significant correlation was seen between RH and either the weighted or unweighted UniFrac distance from indoor to outdoor air (P > 0.05, **Figure 2**).

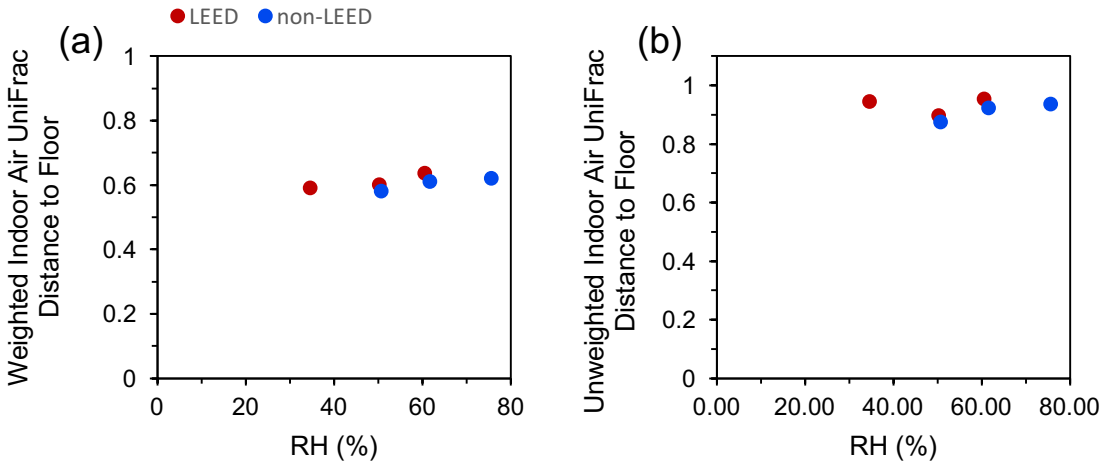

Figure 2: (a) Weighted and (b) unweighted UniFrac distance of indoor to outdoor air compared to mean relative humidity (RH). (a) R = 0.7, P = 0.1. (b) R = 0.2, P = 0.8.

We also used the indoor-to-outdoor weighted UniFrac distance to consider similarity in bacterial communities. "Distance" will be smaller for more similar communities. This distance for LEED buildings

in pairs 2 and 3 was found to be higher than that of corresponding non-LEED, whereas the opposite was found in pair 1 (**Figure 3a**). The unweighted UniFrac distance from indoor to outdoor air was greater for LEED than non-LEED in pairs 1 and 3, but lower in pair 2 (**Figure 3b**).

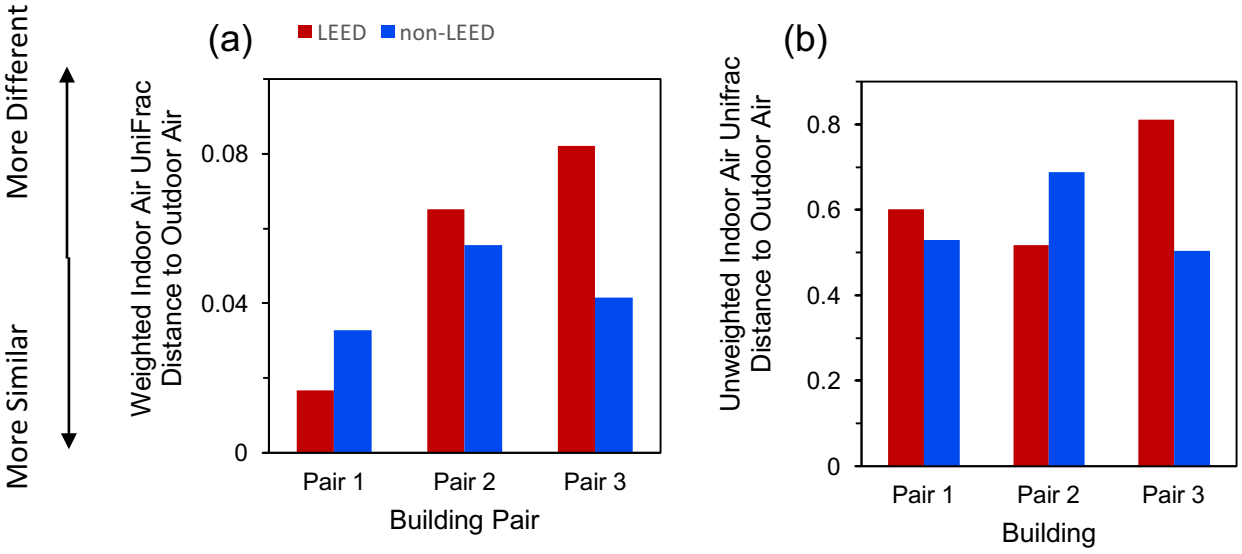

Figure 3: (a) Weighted and (b) unweighted UniFrac distance comparisons of indoor to outdoor air. (a) R = 0.3, P = 0.6. (b) R = 0.3, P = 0.5.

The weighted UniFrac distance from indoor air to carpet dust for both LEED and non-LEED buildings were statistically the same for all pairs (**Figure 4**).

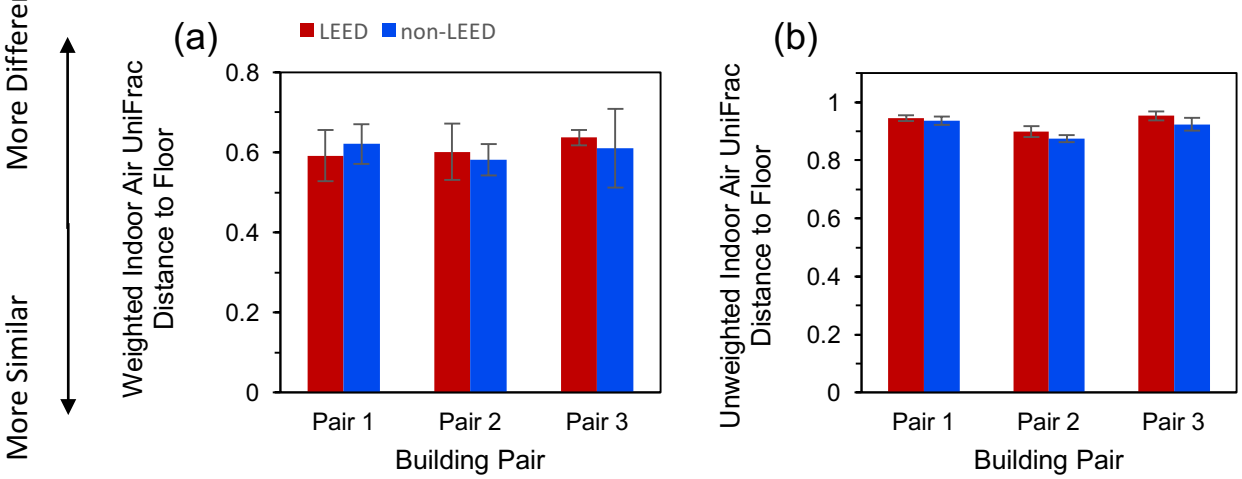

Figure 4: (a) Weighted and (b) unweighted average UniFrac distance comparisons of indoor air to carpet dust. (a) R = 0.008, P = 0.97. (b) R = 0.3, P = 0.2.

In building pairs 1 and 2, dust from entrance carpet was more similar to backroom carpet in non-LEED buildings determined by both the weighted and unweighted UniFrac distance. Building pair 3 demonstrated more similarity between entrance and backroom carpets in LEED (**Figure 5**).

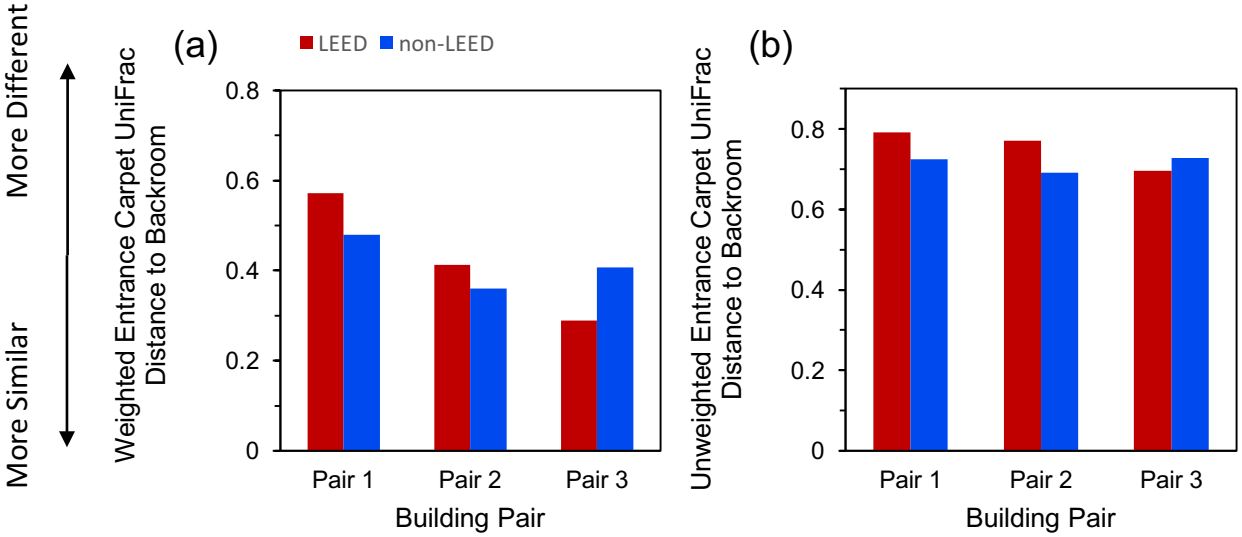

Figure 5: (a) Weighted and (b) unweighted UniFrac distance comparisons of entrance to backroom carpet. (a) R = 0.05, P = 0.9. (b) R = 0.3, P = 0.6.

The principal coordinate plots in **Figure 6** demonstrate the weighted and unweighted UniFrac distance for all air samples. Based on weighted UniFrac, buildings appear to separate by building pairs.

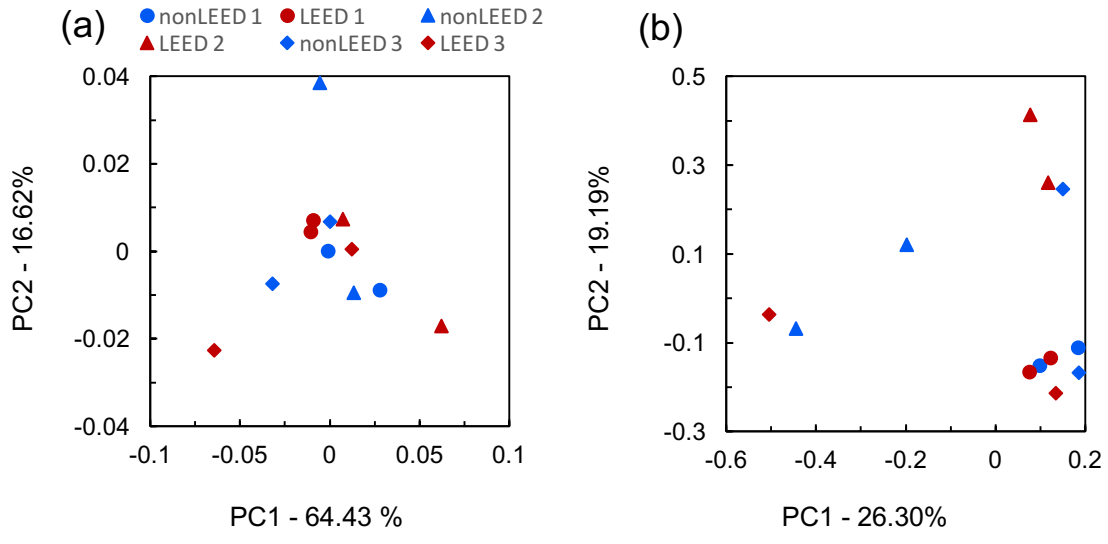

Figure 6: (a) Weighted and (b) unweighted UniFrac PCoA for all air samples.

Principal coordinate analyses of weighted and unweighted UniFrac distances for all samples are shown in **Figure 7**. Air samples were distinctly grouped separate from carpet samples in both the weighted and unweighted plots. In both weighted and unweighted PCoAs, non-LEED carpet samples were grouped closer to one another and closer along the horizontal axes (PC1) to air samples than LEED carpet samples.

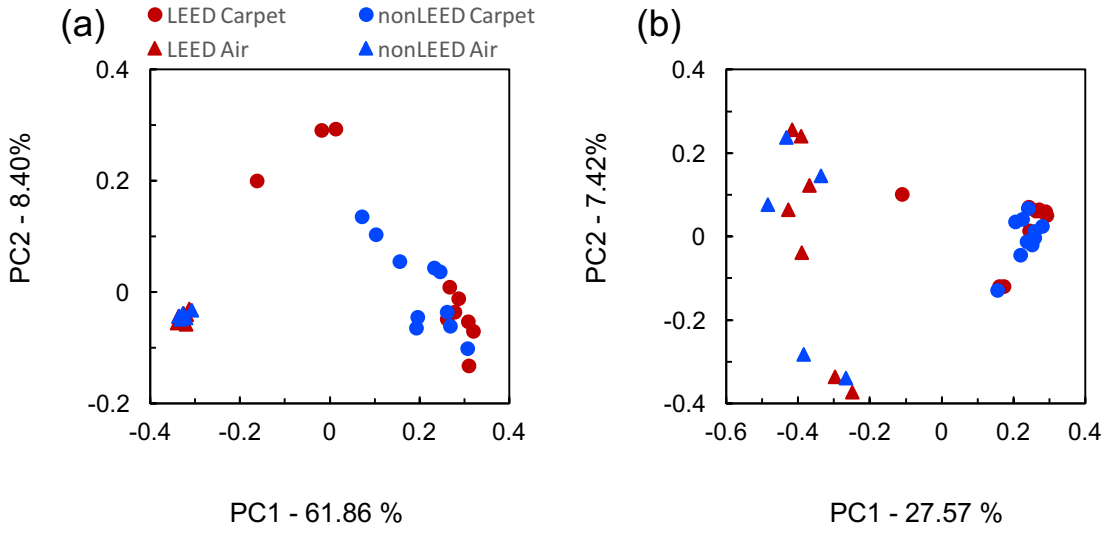

Figure 7: (a) Weighted and (b) unweighted UniFrac PCoA for all air and carpet samples.

Fungal Sequencing

A total of 202098 fungal were included in this analysis. We used the Morisita-Horn distance for fungi to compare community similarities. Fungal communities in LEED carpets were more similar between entrance and backroom than in non-LEED for building pairs 1 and 2 (**Figure 8**).

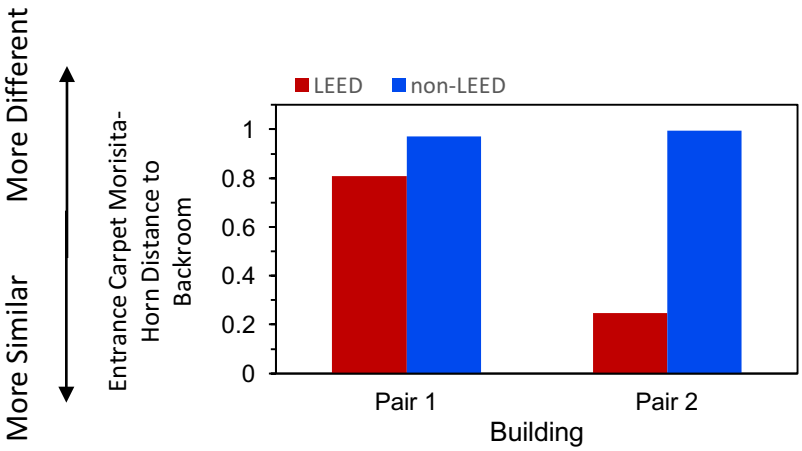

Figure 8: Morisita-Horn distance comparison of entrance to backroom carpet. Insufficient sequencing data prevented from the reliable enumeration of the Morisita-Horn distance from entrance to backroom carpet in building pair 3. R = -0.8, P = 0.2.

LEED carpet samples are grouped closely together in Morisita-Horn principal coordinate analyses, whereas non-LEED carpet samples are scattered across both axes (**Figure 9**). The only air sample able to be included in fungal diversity analyses is indicated by a triangle in the upper right corner of **Figure 9**. Others contained too few reads for inclusion. Although communities in non-LEED carpet samples are more diverse amongst themselves compared to LEED, fungal communities in non-LEED carpet are closer to those of air.

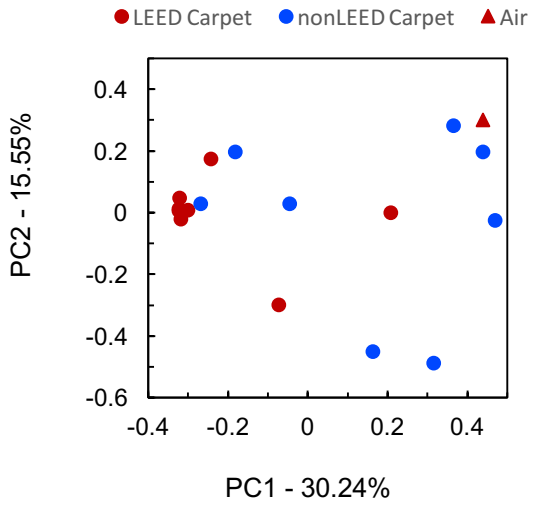

Figure 9: Morisita-Horn PCoA for all air and carpet samples. Of the 32 sequenced samples, 4 did not pass quality trimming. Only 17 subsamples of the remaining 28 were considered for this PCoA due to low OTU counts. Of the 17 samples included in Morisita-Horn PCoA, there was only one air sample (b).

Discussion

Meadow et al. determined in 2014 that microbial communities in indoor air are more similar to outdoor air with improved ventilation irrespective of occupancy.[6] In our study of 3 LEED-certified and 3 non-LEED buildings on the Ohio State University campus, ventilation in LEED buildings was not significantly different from that of non-LEED (**Table 1**). However, the range of measured ventilation values was somewhat narrow. For this reason no indirect, linear correlation was seen between a building's ventilation rate and its microbial communities in air or carpet dust as demonstrated in other studies (**Figure 1**). Correlations between AER and UniFrac distance were not significant ($P > 0.05$). Relative humidity also showed no significant correlation to LEED-certification ($R = 0.6$, $P = 0.2$, $P > 0.05$).

In general, airborne bacterial communities in non-LEED buildings were more similar to that of outdoor air than LEED for both weighted and unweighted UniFracs. However, results were not consistent for all building pairs in either weighted or unweighted UniFrac distance comparisons (**Figure 2**). Bacterial communities in non-LEED carpets were consistently more similar to all related air samples than in LEED for the unweighted UniFrac. This was also the case in 2 of 3 building pairs for weighted UniFrac distance comparisons. Standard deviation error bars negate any statistical significance of these differences (**Figure 3**). Building pairs 1 and 2 consistently demonstrated more similarity between entrance and backroom carpet bacterial communities for non-LEED buildings than for LEED in both weighted and unweighted UniFrac comparisons. Building pair 3 demonstrated more diversity between entrance and backroom carpet bacterial communities for non-LEED buildings than for LEED in both comparisons (**Figure 4**). No significant correlations between LEED-certification and UniFrac distances were observed ($P > 0.05$).

Fungal communities in non-LEED entrance and backroom carpet samples were more different than in LEED, demonstrated by the Morisita-Horn distance comparison (**Figure 7**). LEED buildings

demonstrated a greater difference in air versus carpet fungal communities than non-LEED. But, only one air sample was included in distance comparisons for fungal communities due to low OTU reads. Fungal communities did not show a significant correlation to LEED-certification ($P > 0.05$).

We observed variation in the data. The absence of clear trends in some cases may be due to the fact that buildings are complex systems with many variables that we were not able to measure in this study. However, the clearest trends were seen with building type. Microbial communities in similar buildings clustered together regardless of LEED-certification status. That is, communities in a given building were more similar to those of its pair, chosen for similar building characteristics, than buildings with different occupancy, floor space, or use.

Ventilation source, or strategy, has been seen to have the largest influence on indoor bioaerosols, followed by ventilation rate and relative humidity.[11] However, since all sampled buildings employed mechanical ventilation, ventilation rate and relative humidity are expected to have the largest impact on airborne microbial communities. These trends were not seen in this study.

Limitations

Limitations of this study include the limited number of buildings and small geographic range from selecting only 6 buildings on the Ohio State University campus. Continued sampling across a wider geographic range would provide a larger sample size to increase confidence in results. The narrow range of ventilation rates also made it difficult to establish the relationships shown between ventilation and microbial communities in other studies.[6] Taxonomic analyses not yet complete may provide additional insight into any noted differences between microbial communities in LEED and non-LEED buildings.[12] This study does not yet include any statistical analyses of its results.

Conclusion

No significant differences were seen in ventilation rates between LEED and non-LEED buildings. This indicates that the ventilation rate is not likely responsible for any notable differences between

microbial communities in LEED and non-LEED buildings. The largest difference seen in microbial communities was between air versus carpet samples in all buildings. Some differences were present in LEED versus non-LEED buildings in both fungal and bacterial communities; however, the current results do not describe how these communities differ taxonomically. Taxonomic assignment of OTUs and statistical analyses must be performed before definite statements can be made concerning the differences, if any, between microbial communities in LEED-certified and non-LEED buildings.

Acknowledgements

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References

1. UGBC. *About|LEED*. Retrieved from http://www.usgbc.org/about/leed
2. USGBC. (2015). LEED v4 for Building Design and Construction.
3. Fisk, W. J. (2000). Health and Productivity Gains from Better Indoor Environments and their Relationship with Building Energy Efficiency. *Annual Review of Energy and the Environment*, *25*, 537–66.
4. Meadow, J. F., Altrichter, A. E., Kembel, S. W., Kline, J., Mhuireach, G., Moriyama, M., Northcutt, D., O'Connor, T.K., Womack, A.M., Brown, G.Z., Green, J.L., & Bohannan, B. J. M. (2014). Indoor airborne bacterial communities are influenced by ventilation, occupancy, and outdoor air source. *Indoor Air*, *24*(1), 41–48.
5. Sundell, J., Levin, H., Nazaroff, W. W., Cain, W. S., Fisk, W. J., Grimsrud, D. T., Gyntelberg, F., Li, Y., Persily, A.K., Pickering, A.C., Samet, J.M., Spengler, J.D., Taylor, S.T., & Weschler, C. J. (2011). Ventilation rates and health: Multidisciplinary review of the scientific literature. *Indoor Air*, *21*(3), 191–204.
6. Meadow, J. F., Altrichter, A. E., Kembel, S. W., Kline, J., Mhuireach, G., Moriyama, M., Northcutt, D., O'Connor, T.K., Womack, A.M., Brown, G.Z., Green, J.L., & Bohannan, B. J. M. (2014). Indoor airborne bacterial communities are influenced by ventilation, occupancy, and outdoor air source. *Indoor Air*, *24*(1), 41–48.
7. Belanger, K., Beckett, W., Triche, E., Bracken, M. B., Holford, T., Ren, P., McSharry, J., Gold, D.R., Platts-Mills, T.A.E., Leaderer, B. P. (2003). Symptoms of wheeze and persistent cough in the first year of life: Associations with indoor allergens, air contaminants, and maternal history of asthma. *American Journal of Epidemiology*, *158*(3), 195–202.
8. Belanger, K., Holford, T. R., Gent, J. F., Hill, M. E., Kezik, J. M., & Leaderer, B. P. (2013). Household levels of nitrogen dioxide and pediatric asthma severity. *Epidemiology (Cambridge, Mass.)*, *24*(2), 320–330.
9. Asadi, E., Da Silva, M. C. G., & Costa, J. J. (2013). A systematic indoor air quality audit approach for public buildings. *Environmental Monitoring and Assessment*, *185*(1), 865–875.
10. Larena, I., Salazar, O., González, V., Julián, M. C., & Rubio, V. (1999). Design of a primer for ribosomal DNA internal transcribed spacer with enhanced specificity for ascomycetes. *Journal of Biotechnology*, *75*(2-3), 187–194.
11. Kembel, S. W., Jones, E., Kline, J., Northcutt, D., Stenson, J., Womack, A. M., … Green, J. L. (2012). Architectural design influences the diversity and structure of the built environment microbiome. *The ISME Journal*, *6*(8), 1469–79.
12. Dannemiller, K. C., Gent, J. F., Leaderer, B. P., & Peccia, J. (2015). Influence of housing characteristics on bacterial and fungal communities in homes of asthmatic children. *Indoor Air*, 1–14.

Appendices

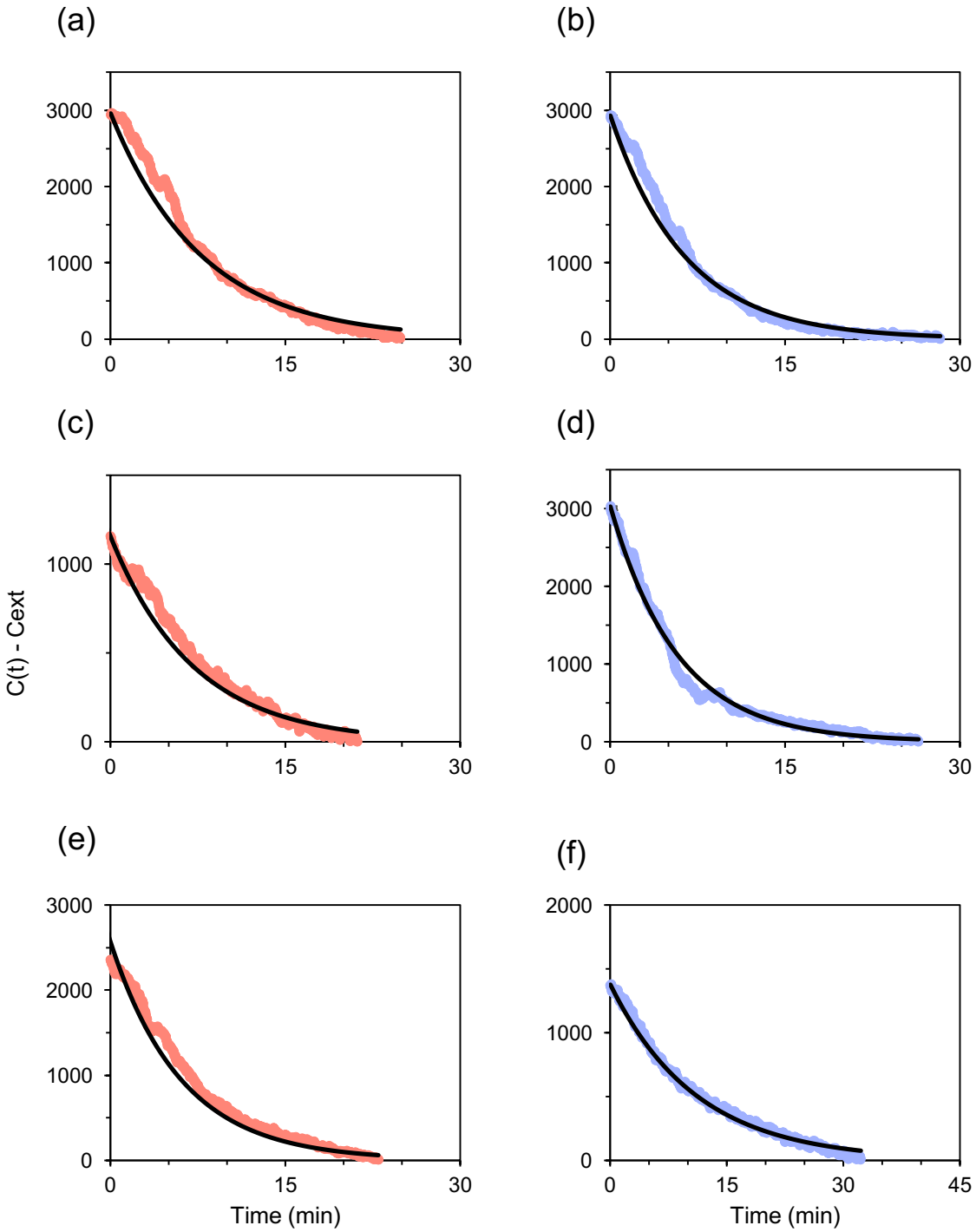

Figure 10: CO_2 curves from AER experiments for all buildings. (a-b) Pair 1. (c-d) Pair 2. (e-f) Pair 3. (a, c, e) LEED buildings. (b, d, f) Non-LEED buildings. Black lines represent trendlines based on calculated AERs.

Table 2: All air sampling collection data

	Building Type	Location	Start Date	Filter Weight (g)	Filter + Dust Weight (g)	Flow (L/min)	Time (min)	Air (L)
Pair 1	LEED	Indoor	8/4/16	0.02066	0.02059	3.93	1511	5934
		Outdoor	8/4/16	0.02065	0.02059	4.10	1504	6170
	nonLEED	Indoor	8/4/16	0.02055	0.02069	4.92	1434	7050
		Outdoor	8/4/16	0.02052	0.02088	4.22	1432	6048
Pair 2	LEED	Indoor	8/11/16	0.02077	0.02098	3.92	1397	5478
		Outdoor	8/11/16	0.02076	0.02106	4.00	1449	5796
	nonLEED	Indoor	8/11/16	0.02059	0.02089	4.46	1405	6271
		Outdoor	8/11/16	0.02079	0.02116	3.23	1426	4603
Pair 3	LEED	Indoor	10/20/16	0.02062	0.02064	4.06	1397	5667
		Outdoor	10/20/16	0.02060	0.02049	3.54	1402	4965
	nonLEED	Indoor	10/20/16	0.02068	0.02068	4.02	1390	5592
		Outdoor	10/20/16	0.02072	0.02066	4.02	1412	5683

Table 3: All carpet dust sampling collection data; *if less than 500 mg of dust was collected, approximately one-half of the available amount was used for DNA extraction.

	Building Type	Location	Date	Thimble Weight (g)	Thimble + Dust Weight (g)	Extraction Quantity (mg)
Pair 1	LEED	Entrance	8/4/16	1.633	2.328	276.54
			8/5/16	1.911	2.439	252.47
		Backroom	8/4/16	1.583	3.377	251.9
			8/5/16	1.557	3.156	253.58
	nonLEED	Entrance	8/4/16	1.644	2.456	251.81
			8/5/16	1.619	1.967	262.6
		Backroom	8/4/16	1.530	5.086	242.05
			8/5/16	1.880	3.983	248.68
Pair 2	LEED	Entrance	8/11/16	1.804	4.103	248.53
			8/12/16	1.599	3.182	251.32
		Backroom	8/11/16	1.674	3.624	255.59
			8/12/16	1.974	2.359	147.62*
	nonLEED	Entrance	8/11/16	1.715	2.989	253.44
			8/12/16	1.641	3.842	256.4
		Backroom	8/11/16	2.049	2.147	28.06
			8/12/16	1.247	1.364	14.5*
Pair 3	LEED	Entrance	10/20/16	1.687	4.287	252.87
		Backroom	10/21/16	1.551	2.028	86.96*
	nonLEED	Entrance	10/20/16	1.979	6.950	245.64
		Backroom	10/21/16	1.636	4.145	266.96

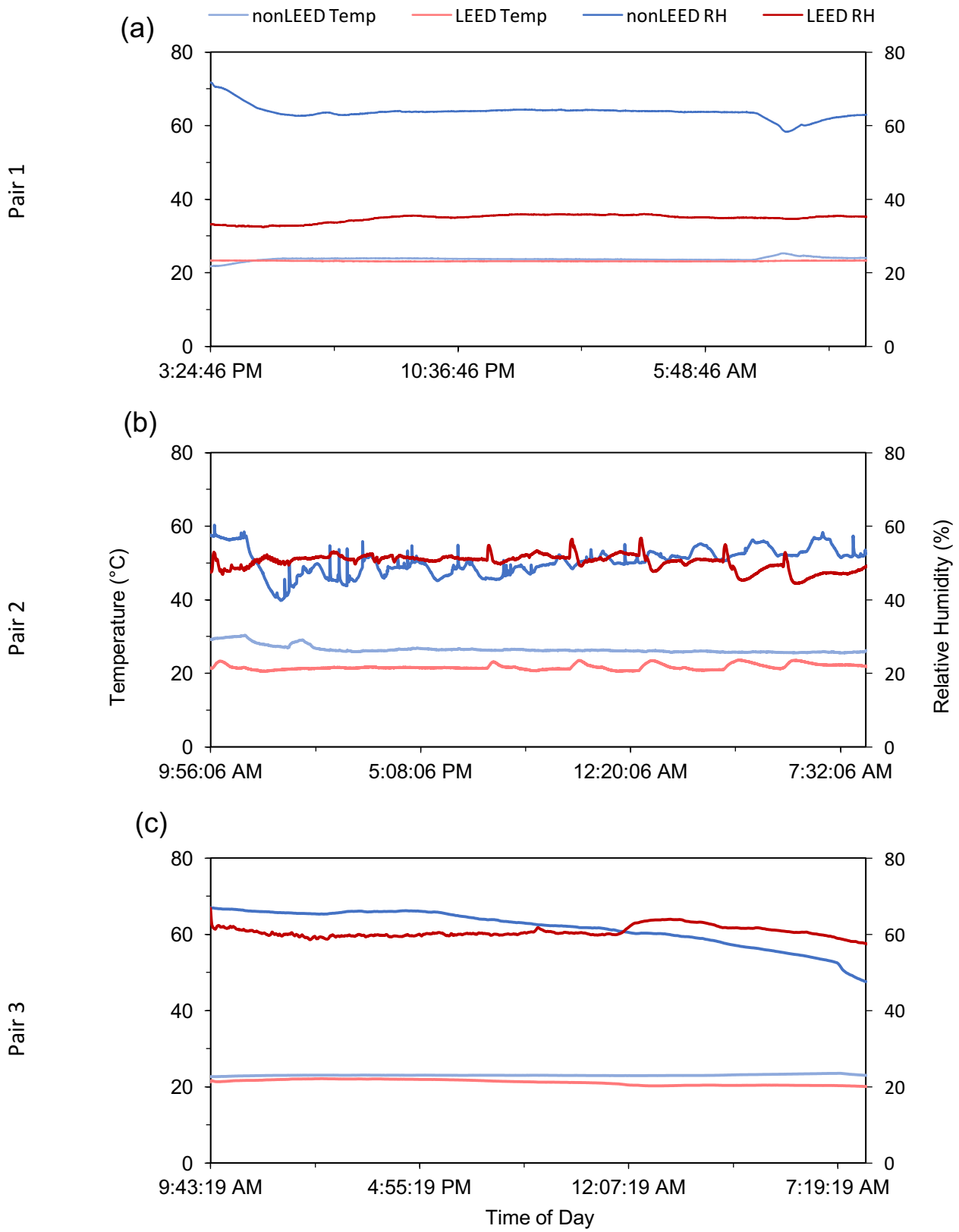

Figure 11: Indoor relative humidity and temperature during air sampling for building pairs 1-3 (a-c).